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Island Life Shapes the Physiology and Life History of Eastern Bluebirds (*Sialia sialis*)*

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ABSTRACT

Island organisms face a range of extrinsic threats to their characteristically small populations. Certain biological differences between island and continental organisms have the potential to exacerbate these threats. Understanding how island birds differ from their continental relatives may provide insight into population viability and serve as a predictive tool for conservation efforts. We compared an eastern bluebird population in Ohio with a threatened population in Bermuda in terms of the birds' development, morphology, immunology, and reproduction. These comparisons revealed that island nestlings had shorter wings and island adults had longer wings than their continental analogs. Island nestlings also had shorter tarsi than continental nestlings at day 8 posthatch, but this difference was absent at day 15 and in adults. Adults weighed less in Bermuda than in Ohio, and both nestlings and adults in Bermuda exhibited higher levels of two immunological indexes (concentrations of an acute-phase protein and titers of nonspecific antibodies). Clutch sizes and hatch rates did not differ between the island and continental populations; however, as the breeding season progressed, brood sizes declined in Bermuda, whereas no such decline occurred in Ohio. Despite these differences and differences in nestling development, island and continental parents fed their nestlings at equal rates. Overall, our results suggest that the Bermuda phenotype may be adjusted to certain aspects of the island environment but not to others. Efforts to conserve the bluebirds of Bermuda may be improved by focusing on the intraseasonal patterns in nestling mortality and, more generally, the survival probabilities of different age classes.

Introduction

Island organisms tend to diverge from their closest continental relatives in terms of their morphology, demography, physiology, and behavior (Foster 1964; Case 1978; Adler and Levins 1994; Grant 1998; Clegg and Owens 2002; Lomolino 2005; Mathys and Lockwood 2009). Efforts to better understand this divergence have resulted in generalities concerning the direction of change. The "island rule" predicts that small species should increase in size when isolated on islands (e.g., Mathys and Lockwood 2009), while the "island syndrome" relates an array of changes including increased body size, slower maturation, and reduced reproductive output (Adler and Levins 1994). These changes are partially the product of ecological differences between islands and continents. For example, an absence of native predators on islands might mean that some island populations have experienced reduced selection pressures compared to some continental populations (Adler and Levins 1994). Island organisms are also frequently characterized by small population sizes and limited geographical distributions (Lack 1942; Paulay 1994). As such, island populations are at particular risk of decline from a variety of factors that can range from the systematic (e.g., urbanization; Suarez-Rubio and Thomlinson 2009) to the stochastic (e.g., a single weather event; Spiller et al. 1998). Certain biological differences between island and continental organisms have the potential to exacerbate this risk. Differences in life history strategy that equate to reductions in reproductive output (e.g., fewer offspring; Adler and Levins 1994) likely fall into this category, and differences in physiology that equate to increases in liability to disease might as well (Warner 1968; Wikelski et al. 2004; Gangoso et al. 2009; Alcaide et al. 2010). In general, with island extinctions far outnumbering continental ones (Kingsford et al. 2009; Loehle and Eschenbach 2012), the extinction record seemingly reflects the exceptional hazards faced by island organisms.

Understanding how island birds differ from their closest continental relatives in terms of parameters that may affect population viability can serve as a predictive tool for conservation efforts, much in the same way that baseline physiological data are useful to managers for recognizing or ruling out potential problems (Wikelski and Cooke 2006). This understanding can

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come through systematic pairwise comparisons of sister groups (e.g., populations or species) that are found on isolated oceanic islands and continents, an approach that bypasses the effects of phylogenetic history. For example, an investigation of 15 island-continent pairs of birds revealed that some immunological indexes were significantly, or at least consistently, higher in the island form while other indexes did not differ (Matson 2006). Thus, island life apparently leads to an immunological reorganization; however, little can be concluded regarding links between this reorganization and disease susceptibility or other aspects of avian biology (Matson 2006). Other studies focused on single island-continent pairs (e.g., Egyptian vulture, *Neophron percnopterus* [Gangoso et al. 2009] and Eurasian kestrel, *Falco tinnunculus* [Alcaide et al. 2010]) did reveal differences in terms of both immune and disease parameters. Thus, data support the notion that the evolutionary divergence associated with island life relates to the physiology of self-maintenance in birds. Yet comparisons of island and continental bird populations that incorporate parameters of immunology, physiology, morphology, life history, and behavior are currently lacking, even though the conservation value of such data is well recognized (Wikelski and Cooke 2006). With this in mind, we compared island and continental populations of the eastern bluebird (*Sialia sialis*), broadly studying these birds from egg to adult.

The eastern bluebird is ideally suited for this investigation. First, this species meets our island-continent distribution requirement; the eastern bluebird ranges across much of eastern North America and is considered a native resident of Bermuda (Crowell 1962; Lockwood and Moulton 1994; Gowaty and Plissner 1998; but also see Avery et al. 2013). Bermuda is an archipelago that totals ~54 km^{-2} and lies in the North Atlantic ~1,100 km off the eastern coast of the United States. Second, the eastern bluebird is relatively well studied. From descriptions of its natural history to examinations of its ecology, physiology, and behavior, diverse aspects of the biology of the eastern bluebird have been documented in a range of populations (reviewed in Gowaty and Plissner 1998). In the United States, the species is not threatened, and populations have increased after several decades (during the 1960s and 1970s) of decline (Sauer and Droege 1990; Gowaty and Plissner 1998). Less well studied, the Bermuda population has been categorized as declining (Nice and Bourne 1958), threatened (Gowaty and Plissner 1998), and vulnerable (Bermuda Biodiversity Project 2007), and that last report specifies the need for "research and monitoring programmes for eastern bluebirds" in Bermuda. More generally, investigations of eastern bluebirds have flourished thanks in part to the species' ready use of nest boxes, which facilitates observation and capture of adult bluebirds at predictable and consistent stages of breeding. Additionally, nestlings in nest boxes can be easily and repeatedly observed, measured, and sampled. Managed nest box networks exist throughout the species' breeding range in North America and Bermuda. Overall, the eastern bluebird is uniquely well suited for exploring how island life affects birds and how these effects may be useful for predicting population declines and shaping conservation measures.

We quantified multiple parameters related to the biology of eastern bluebirds in Bermuda, and we compared these parameters to similarly measured conspecifics from a population in the continental United States. Our goal was twofold. First, we worked to describe the bluebirds in order to establish baseline data on two distinct populations living in very different environments. Second, we attempted to identify island-continent differences that, either individually or as part of a broader phenomenon, hold the potential to intensify the risks of decline that are already associated with a small and geographically isolated population, such as the one in Bermuda. We accomplished our goal by similarly analyzing four data sets, which were organized according to fundamental attributes of the data (e.g., life history stage, nested structure of the data, etc.) rather than specific biological queries. Two data sets concern morphological and immunological indexes: the first relates to absolute levels and developmental states in nestlings; the second relates to absolute levels in adults. Two other data sets concern reproduction: the first includes life history parameters, such as the number of eggs and nestlings per nest; the second centers on food delivery, a nest-level parental behavior with clear consequences for nestling growth and development.

We asked the following two questions, which served to interweave our four data sets: (1) Do island and continental bluebird populations differ in their development, morphology, immunology, and reproduction? (2) When taken together, do the population differences suggest intensified risks of decline in the island population? In light of these questions and the generalized changes associated with small animals living on isolated islands (i.e., in line with the island rule and island syndrome), we could make several predictions. We expected the eastern bluebirds of Bermuda to develop more slowly but to eventually reach a larger size (but see Grant 1965; Meiri et al. 2008). Making directional predictions regarding immune function is more challenging. If disease pressures are lower on islands, immune defenses might be reduced. Alternatively, if island life indeed allows for slower development or favors longevity, immune defenses might be intensified (as in Matson 2006). We expected the eastern bluebirds of Bermuda to produce fewer eggs and young. Reduced reproductive output combined with slower development and larger size would suggest a broad shift in life history. This type of shift in life history might be accompanied by reduced rates of food delivery by island parents compared with continental parents. Mismatches (i.e., biologically relevant incongruities with fitness implications), either between a population's life history and physiology (e.g., reduced reproduction and reduced self-maintenance) or between its life history and environment (Blomberg et al. 2012), will be useful for forecasting population trends and directing conservation efforts.

Material and Methods

Study System

We studied eastern bluebirds in the continental United States in 2008 and 2009 and on the islands of Bermuda in 2010 and 2011. In the United States, we studied birds in north central

Ohio (ca. 40°52′N, 82°19′W). This area is near the center of this species' continental distribution. In Bermuda (ca. 32°20′N, 64°45′W), we studied birds in the two easternmost parishes.

All fieldwork was preceded by regular nest box inspections by local volunteers, and in both locations, the first eggs were typically reported in mid-April. The systematic field investigations of adults and their nestlings were timed to coincide with the first peak in hatching and thus began around May 1 in all years. Fieldwork ended in June (2009–2011) or July (2008 only). All work was approved by the relevant institutional animal use committees (at Kenyon College, the College of Wooster, and Bermuda Institute of Ocean Sciences) and permitted by the pertinent government agencies (i.e., Ohio Department of Natural Resources, US Fish and Wildlife Services, and Bermuda Department of Conservation Services).

Data and Blood Sample Collection

We visited nest boxes with sufficient regularity to pinpoint hatching to the day. Additionally, we recorded the maximum number of eggs in each nest. On days 2, 4, 8, and 15 posthatch, we measured the body mass, the length of the flattened right wing, and the length of the right tarsus of each nestling. We caught the parent bluebirds once on day 12 (± 1 d) posthatch and recorded the same morphological measurements. Accordingly, we were able to analyze the nestling measurements in terms of both size (i.e., absolute mass or length) and development (i.e., % of size at maturity). We calculated development by dividing a measurement of a chick by the mean value of the same measurement of the adults in the same location (e.g., mass Ohio nestling$_i$/mean mass Ohio adults $\times$ 100). From each nestling (on days 8 and 15 posthatch) and from each captured adult, we collected a blood sample (always equivalent to <1% of body mass, typically between 200 and 250 μL) from the brachial vein into heparinized capillary tubes. The whole blood was centrifuged to separate it into plasma and packed erythrocyte fractions, which were stored frozen.

Immunological Indexes

We analyzed the plasma samples using two immunological assays. Since standardized versions of both assays are routinely used and published by us and others (e.g., Mauck et al. 2005; Palacios et al. 2007; Buehler et al. 2009; Cellier-Holzem et al. 2010; van de Crommenacker et al. 2010; Berzins et al. 2011; Hegemann et al. 2012; Horrocks et al. 2012*a*; Matson et al. 2012*a*), we provide only basic information here.

First, we measured concentrations (mg mL^{-1}) of plasma haptoglobin (Quaye 2008) using a commercially available colorimetric assay (TP801; Tridelta Development, Maynooth, County Kildare, Ireland; Matson et al. 2012*b*). Ten haptoglobin assay plates used in this study included an among-plate standard, which was run in duplicate within each plate (mean among-plate coefficient of variation [CV] = 15%; mean within-plate CV = 10%).

Second, we quantified plasma titers of complement-like lytic enzymes (via lysis) and nonspecific natural antibodies (via agglutination) using a hemolysis-hemagglutination assay with rabbit red blood cells (RBA050; HemoStat Laboratories, Dixon, CA; Matson et al. 2005). Scanned images of individual serially diluted samples were randomized among all plates and scored blindly to sample identity (by K.D.M.). Strong effects of the age of sample batches and very low titers prohibited meaningful analysis of the effects of location on lysis. (Further details are provided in the appendix, available in the online edition of *Physiological and Biochemical Zoology*.) The 119 hemolysis-hemagglutination assay plates used in this study included an among-plate standard (chicken plasma), which was run in duplicate within each plate (agglutination: mean among-plate CV = 16%; mean within-plate CV = 9%).

Agglutination reflects the circulating level of nonspecific natural antibodies and gauges a facet of innate immunity that is, in general, temporally stable (Matson et al. 2005; but see Hegemann et al. 2012). Haptoglobin is a heme-binding acute-phase protein with multiple physiological functions (Quaye 2008), including preventing free heme from serving as a nutrient for infectious microorganisms (Eaton et al. 1982). Compared to agglutination titers, haptoglobin concentrations are considerably more labile over short timescales (van de Crommenacker et al. 2010; Matson et al. 2012*a*, 2012*b*). A growing body of research explores both indexes in the contexts of avian ecology (Hegemann et al. 2012; Horrocks et al. 2012*b*), life history (Palacios et al. 2007; Versteegh et al. 2012), and geography (Matson 2006; Horrocks et al. 2012*a*).

Behavioral Observations

We counted the number of feeding trips made by males and females to each nest box during a 1- to 2-h period in late morning (mean observation midpoint ~10:40 a.m.) on day 12 (± 1 d) posthatch. A feeding trip was defined as an adult male or female bluebird flying to the nest box with food in its bill and placing its head into the box. Observations were never preceded on the same day by a nest visit, a parental capture attempt, or any other research activity involving the focal nest.

Statistical Analyses

We conducted all analyses in the R programming environment (ver. 2.15.0; R Development Core Team 2012). Our central statistical goal was to identify whether location (continent vs. island) was important in explaining variation in each dependent variable. Accordingly, we contrasted three hierarchical models using an information-theoretic approach (Burnham and Anderson 2002), as follows: (1) a model with no location terms (hereafter, the basic model), (2) the basic model with the addition of main effect of location (the location model), and (3) the location model with the addition of all biologically relevant two-way interactions involving location (the location-interaction model). For each dependent variable, we ranked the three models based on ΔAIC_c (package "AICcmodavg"; Mazerolle 2012). We identified the best of the three models (the final

model) using the following two guidelines: (1) In cases when the second-ranked model had $\Delta AIC_c > 2$, the model with the lowest AIC_c served as the final model. (2) In cases when two or three models were separated by $\Delta AIC_c < 2$, the most parsimonious (i.e., least parameterized) model served as the final model. When either the location model or the location-interaction model was identified as the final model, we report the germane location term statistics, which we derived from a post hoc analysis of that final model (Mauck et al. 2005). Though tested and reported when significant, interactions involving location were not subject to a priori predictions and were primarily included as a means to robustly evaluate the overall importance of location.

For the variables concerning nestling size and development, adult characteristics, and parental feeding behavior, we used linear mixed models ("lme" in package "nlme"; Pinheiro et al. 2012) with a random term that reflected the nested nature of the data (i.e., nestlings within nests, repeated measurements within individual adults, or pairs of parents within territories). For egg and nestling counts, we used generalized linear mixed models ("lmer" in package "lme4"; Bates et al. 2012). Here, we set the model family to Poisson, and we included the identity of the nest box as a random factor. Continuous covariates (e.g., Julian dates, initial [day 2] masses, etc.) were standardized before their use by calculating the residuals of a model that included location as the sole explanatory variable. We confirmed that dependent variables were adequately described by a Gaussian or Poisson distribution via graphical and statistical methods. In marginal cases, we improved normality through data transformation, a process that had limited effects on the statistical outcomes. Thus, in most cases we present results based on the untransformed data. Sample sizes are provided in table 1. Information on specific methodological covariates and data transformations is noted, along with other data set– or dependent variable–specific details, in the appendix.

Table 1: Sample sizes

	Bermuda		Ohio	
Parameters and time point	n_{obs}	n_{groups}	n_{obs}	n_{groups}
Nestling:				
Development/ morphology:				
Day 8	67	21	90	25[a]
Day 15	43	14	82	24[a]
Haptoglobin:				
Day 8	64	20	80	22[a]
Day 15	38	12	66	19[a]
Agglutination:				
Day 8	64	20	75	22[a]
Day 15	38	12	65	19[a]
Adult:[b]				
Morphology and haptoglobin	35	34	63	50[c]
Agglutination	35	34	57	46[c]
Clutch and brood sizes:				
Days 0 and 2	42	33	55	38[d]
Day 15	25	21	33	25[d]
Feeding behaviors	27	24	34	25[d]

[a]Nestlings within nests.
[b]Among total observations: Bermuda = 17 male, 18 female; Ohio = 29 male, 35 female.
[c]Pairs of parents within territories.
[d]Repeated measurements within individual adults.

Results

Nestlings

Morphology. Nestlings from Bermuda and Ohio differed in terms of their size and development. Variables related to the wings of nestlings were consistently different between the populations. Specifically, the location model was identified as the final model for wing length and wing development at days 8 and 15 posthatch (table A1, available as a PDF in the online edition of *Physiological and Biochemical Zoology*). The location model for wing variables always included initial nestling mass and the Julian date of hatch. The post hoc analyses revealed a highly significant effect of location for all dependent variables related to nestling wing length (table A1). Compared to the wings of similarly aged nestlings in Ohio, the wings of nestlings in Bermuda were shorter (e.g., day 15: Bermuda = 59.7 mm, Ohio = 63.3 mm; $t_{36} = 4.46$, $P < 0.001$) and less developed (e.g., day 15: $t_{36} = 5.52$, $P < 0.001$; fig. 1*A*; table A1).

Variables related to the tarsi of nestlings differed between the Bermuda and Ohio populations at 8 d posthatch but not at 15 d posthatch. The location model was identified as the final model for the tarsus length and tarsus development at day 8 (table A1). The post hoc analyses revealed a highly significant effect of location in both cases (table A1). Compared to the tarsi of day 8 nestlings in Ohio, the tarsi of nestlings in Bermuda were shorter (Bermuda = 16.80 mm, Ohio = 17.87 mm; $t_{44} = 3.57$, $P < 0.001$) and less developed ($t_{44} = 3.13$, $P = 0.003$; fig. 1*B*; table A1). For tarsus length and development at 15 d posthatch, we identified the basic model, which included only initial nestling mass and the Julian date of hatch, as the best model (table A1).

Variables concerning the mass of 8- and 15-d-old nestlings (including mass development; fig. 1*C*) showed complex effects of location that were related to initial mass. The location-interaction model was consistently identified as the final model for nestling mass and mass development at both 8 and 15 d posthatch (table A1). In all cases, the interaction between location and initial nestling mass was significant according to the post hoc analyses of the location-interaction models (day 8 mass: $t_{107} = -2.44$, $P = 0.017$; day 8 development: $t_{107} = -2.66$, $P = 0.009$; day 15 mass: $t_{83} = -2.95$, $P = 0.004$; day 15 development: $t_{83} = -3.02$, $P = 0.003$). These significant interactions meant that the correlations between initial mass (on day 2) and subsequent mass (on days 8 and 15) differed depending on whether the nestlings were from the continental or

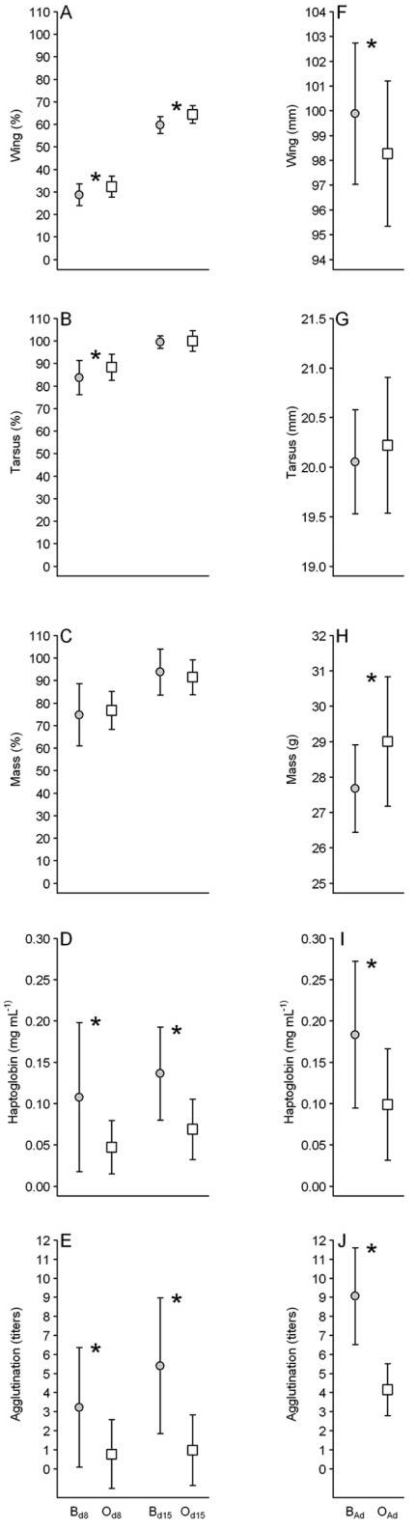

Figure 1. Nestling development indexes (*A–C*) suggest that with some facets of morphology, birds in Bermuda lag behind ones in Ohio. Adult size indexes (*F–H*) show that the birds in Bermuda have longer wings but are lighter than birds in Ohio. Immunological indexes in nestlings (*D*, *E*) and adults (*I*, *J*) reveal consistently higher values in Bermuda compared to Ohio. The symbols (Bermuda = circles, Ohio = squares) represent the mean values of the raw data (i.e., not accounting for any hierarchical structure in the data), and the error bars represent the related standard deviations. Asterisks denote significant ($P < 0.05$) effects of location (and that the location model was identified as the final model). We present nestling morphology measurements (*A–C*) in terms of development (i.e., % of size at maturity), which we calculated by dividing a measurement of a chick by the mean value of the same measurement of the adults in the same location (e.g., mass Ohio nestling$_i$/mean mass Ohio adults × 100). In order to fully plot positive error bars while maintaining a consistent scale, the *Y*-axes for these three subplots always extend to 110%.

island population. Further post hoc analysis led to equivocal results concerning the effect of location alone. For example, after categorizing nestlings as initially light (residual initial mass < 0) or initially heavy (residual initial mass > 0) and analyzing these classes separately, only two of the eight possible tests (four variables, two age classes) revealed results that could be considered statistically significant. After accounting for the Julian date of hatch, those nestlings that were initially heavy (1) weighed more in Ohio than in Bermuda at 8 d posthatch (Bermuda = 20.7 g, Ohio = 22.3 g; $t_{30} = 2.26$, $P = 0.031$) and (2) had reached a smaller percentage of adult mass in Ohio than in Bermuda at 15 d posthatch (Bermuda = 93.8%, Ohio = 91.5%; $t_{25} = 2.23$, $P = 0.035$). No differences between the Bermuda and Ohio populations were uncovered for those nestlings that were initially light.

Immunology. Nestlings from Bermuda and Ohio also differed in terms of immunological indexes. The location model was identified as the final model for haptoglobin concentrations at both 8 and 15 d posthatch (table A1). The related post hoc analyses revealed that at both time points the nestlings from Bermuda exhibited significantly higher concentrations of haptoglobin than the nestlings from Ohio (e.g., day 15: $t_{29} = -6.38$, $P < 0.001$; fig. 1*D*; table A1). In contrast, for the variables related to the ontogeny of haptoglobin (i.e., the percent of the population-specific adult concentration realized by 8-d-old nestlings and by 15-d-old nestlings), the basic model was identified as the final model (table A1).

The location model was also identified as the final model for agglutination titers at both 8 and 15 d posthatch (table A1). The related post hoc analyses revealed that at both time points the nestlings from Bermuda exhibited significantly higher agglutination titers than the nestlings from Ohio (e.g., day 15: $t_{29} = -8.11$, $P < 0.001$; fig. 1*E*; table A1). We did not analyze the ontogeny of agglutination because there were many nonresponders (zero titers) at both time points (further details are provided in the appendix), leading to little detectable change within nestlings.

Adults

Morphology. Adults from Bermuda and Ohio differed morphologically. With wing length and body mass, the location model was identified as the final model (table A1). Post hoc

analyses of the final models suggest that, compared to their counterparts in Ohio, adult bluebirds in Bermuda had significantly longer wings ($t_{81} = -2.83$, $P = 0.006$; fig. 1*F*; table A1) and weighed significantly less ($t_{81} = 3.72$, $P < 0.001$; fig. 1*H*; table A1). In contrast, the basic model for adult morphology, which included sex and the Julian date of the measurement, was identified as the final model for tarsus length (fig. 1*G*; table A1).

Immunology. Adults from Bermuda and Ohio also differed immunologically. With both haptoglobin concentrations and agglutination titers, the location model was identified as the final model (table A1). Post hoc analyses of the final models suggest that, compared to their counterparts in Ohio, adult bluebirds in Bermuda exhibited higher haptoglobin concentrations ($t_{81} = -6.14$, $P < 0.001$; fig. 1*I*; table A1) and higher agglutination titers ($t_{77} = -11.37$, $P < 0.001$; fig. 1*J*; table A1). With these two dependent variables, the location model and the location-interaction model both far outperformed the basic model, which included sex, the Julian date of sample collection, and, in the case of haptoglobin, an index of plasma redness (absorbance at 450 nm) to control for the effects of hemolysis.

Clutch and Brood Sizes

We found no compelling evidence for differences between the Bermuda and Ohio populations with regard to the number of eggs per clutch (Bermuda = 4.4, Ohio = 4.8) or the brood size at 2 d posthatch (Bermuda = 3.6, Ohio = 4.3). With these two variables, the basic model, which included only the Julian date of hatch, best explained the observed variation (fig. 2*A*; table A1).

In contrast, the brood size at 15 d posthatch differed between the Bermuda and Ohio populations later, but not earlier, in the breeding season. Specifically, we identified the location-interaction model as the final model (table A1). In this model, the interaction between location and Julian hatch date was significant ($z = 2.66$, $P = 0.008$; fig. 2*B*). Further post hoc analyses revealed the following: in the first half of the study period (before the median Julian hatch date of 132 [May 12]), the effect of location was not significant (Bermuda = 3.8 nestlings, Ohio = 3.7 nestlings; $z = -0.11$, $P = 0.910$), but in the second half of the study period (on or after the median Julian hatch date of 132 [May 12]), the brood size at 15 d posthatch was significantly smaller in Bermuda (1.8 nestlings) than in Ohio (3.9 nestlings; $z = 3.18$, $P < 0.002$).

Parental Feeding Behavior

We found no compelling evidence for differences in parental feeding behavior between the Bermuda and Ohio populations. The basic model, which included only the Julian date of observation, was chosen as the final model for both the number of feeds per hour per clutch (Bermuda = 12.0, Ohio = 12.9) and the number of feeds per hour per nestling (Bermuda = 4.1, Ohio = 3.3; fig. 2*C*; table A1).

Discussion

The eastern bluebirds of Bermuda differed in a variety of ways from their continental relatives. These differences span life history stages and physiological systems. Compared to continental bluebirds, island nestlings developed more slowly, but island nestlings and adults exhibited higher levels of two immunological indexes. These results provide no evidence for a mismatch between life history and physiology but suggest that an alternate life history strategy, one characterized by increased self-maintenance, might be in place in the Bermuda population. However, differences between the populations in terms of reproduction paint a slightly different picture. Birds in both populations laid and successfully hatched similar numbers of eggs per clutch, but as the breeding season progressed, brood sizes at day 15 declined in Bermuda, while no such decline occurred in Ohio. Since nestling feeding rates were similar, the decline in brood size in Bermuda was apparently not simply a manifestation of differences in parental effort. Our results suggest that some elements of the biology of the bluebirds of Bermuda (e.g., slow development and high immune indexes) may be complementary to each other and to biotic and abiotic features of their island environment. Such congruities offer little in the way of warnings to conservation planners. In contrast, incongruent elements (e.g., seasonal reductions in brood size despite constant parental feeding behaviors) help bring conservation concerns into focus and pinpoint topics needing further study. Overall, we emphasize the need to understand the match between an island organism, its environment, and its evolutionary history in a population-specific context.

Island-Continent Differences in Indexes of Development and Immunology Suggest Divergent Life History Strategies

Bluebird nestlings developed more slowly in Bermuda than in Ohio. Whereas the tarsi of birds in Bermuda appeared to exhibit compensatory growth during the second half of the nestling period, the wings of these birds remained less developed at day 15 posthatch. Moreover, since nestlings had shorter wings and adults had longer wings in Bermuda compared to those in Ohio, differences between the populations in terms of wing growth (e.g., rate, duration, etc.) apparently extended beyond the nestling period. Slower development possibly signals a shift in life history that can be characterized by increased investment in self-maintenance (e.g., as with tropical birds; Ricklefs and Wikelski 2002). Differences in the predation pressures faced by birds inside and outside the nest represent one possible avenue to slower development, with or without this more general shift in strategy (Coslovsky and Richner 2011). In fact, natural predators of bluebirds are reduced in Bermuda (though, at present, bluebirds must cope with feral cats and several introduced bird species; Crowell and Crowell 1976). Further investigations are required to more fully elucidate the role of predators in shaping the phenotype of island bluebirds. Additionally, while we used measurements on day 15 as proxies for the characteristics at

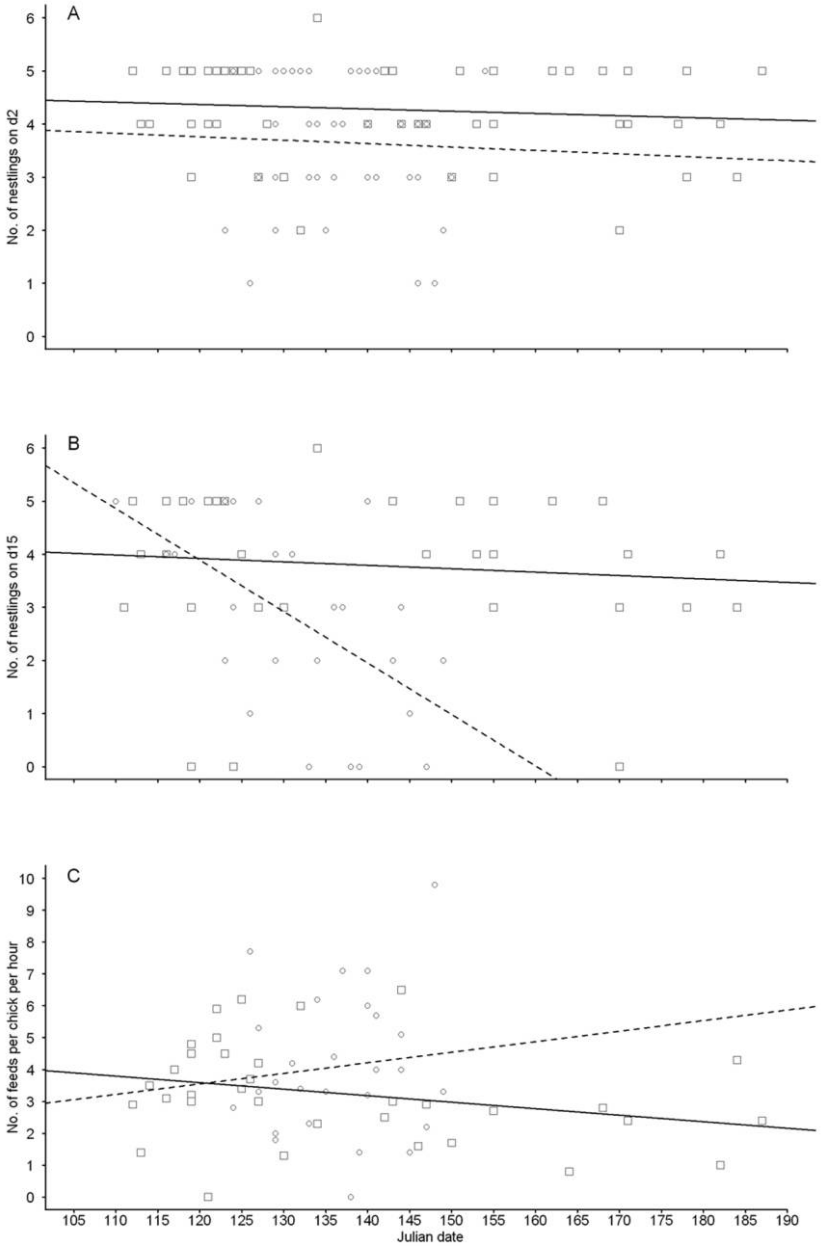

Figure 2. At 2 d posthatch (*A*), the number of nestlings was unrelated to Julian hatch date in Bermuda (dashed line; nests = circles) and Ohio (solid line; nests = squares); at 15 d posthatch (*B*), the number nestlings declined with increasing Julian date in Bermuda but not in Ohio (location by Julian date interaction: $z = 2.66$, $P = 0.008$). The total number of feeding trips (male plus female) per chick per hour (*C*) was unrelated to Julian date in both locations.

fledging (Pinkowski 1975), data on the precise age and developmental state at fledging are needed (Radersma et al. 2011).

Both of the measured immunological indexes were higher in 8- and 15-d-old nestlings in Bermuda. Differences between the populations in terms of life history, pathogen pressure, or both could explain the observed differences in these immunological indexes (Horrocks et al. 2011). For example, island birds might face an immunobiome that is quantitatively or qualitatively different from the one faced by continental birds (Matson 2006; Horrocks et al. 2011). However, for this to drive the observed pattern, the difference would have to exert comparable genetic effects (e.g., selection pressures) on both immunological indexes or impose systematic effects on pre- or neonatal phenotypes (e.g., via mothers [Grindstaff 2008] or directly [Goren et al. 1988]). Because the typically stable index of agglutination is also substantially elevated, it is more challenging to envision a scenario in which the population differences in both immunological indexes are solely the consequence

of an infection with a disease-causing organism (i.e., a result of an acute-phase response). Examining within-nestling changes in haptoglobin is also useful. During the second week of the nestling period, though haptoglobin concentrations differed between populations, the concentrations increased at similar rates, thus raising questions about the ontogeny of haptoglobin in ovo and in young chicks. The simplest explanations are that haptoglobin concentrations in the island birds are higher at hatching, increase more rapidly in the first week, or both. This study also confirms and broadens the island-continent difference in haptoglobin concentrations among adult bluebirds, which was first reported for populations from Bermuda and Illinois (Matson 2006).

Overall, these results suggest a shift in the ecology of bluebirds in Bermuda to a strategy characterized by slower growth and higher levels of immune defenses (Lee et al. 2008). Additional comparative and experimental studies focused on the size and composition of eggs (e.g., Shawkey et al. 2008) and the microbial and physiological ecology of eggs and nestlings (Kohl 2012; Peralta-Sánchez et al. 2012) are needed to further disentangle the relative roles of life history and pathogen pressure in island and continental bluebirds.

Island and Continental Adults Differed Morphologically but Not as Predicted

The adult bluebirds in Bermuda were lighter and had longer wings than the adults in Ohio. These differences contrast with our expectations based on the typical changes associated with small animals living on isolated islands (e.g., the island syndrome and island rule; Adler and Levins 1994; Clegg and Owens 2002; Lomolino 2005; but see Grant 1965; Meiri et al. 2008). For example, individuals in the introduced population of Great kiskadees (*Pitangus sulphuratus*) in Bermuda are heavier and have longer wings than individuals in the native source population on the island of Trinidad (Mathys and Lockwood 2009). Given the ostensible role of predator release in shaping island life, the bluebird and kiskadee results are potentially at odds with the finding that exposure of mothers to predators results in offspring with longer wings at maturity (Coslovsky and Richner 2011). Another mechanism—reduced nutritional resources—might limit adult mass in the island population but would be unlikely to stimulate the growth of wing feathers in the same birds. The lightweight and long-winged birds in our study may simply signify a founder effect: the individuals that made it to Bermuda may have had this morphology, and the population there may have lacked the time or the variation needed to become heavier and achieve a more typical island morphology (but see Mathys and Lockwood 2009). This and other population genetics phenomena, which could also underlie the immunological differences between populations (see above), deserve further study.

Reproduction Influenced by Seasonal Dynamics within Locations but Not Location Alone

Numbers of eggs and hatchlings produced did not differ between island and continental bluebirds. Moreover, feeding rates (per chick and per clutch) were unrelated to the location of the population. These results suggest relatively similar reproductive strategies in the two populations (also see Crowell and Rothstein 1981) and thereby may serve to temper conclusions relating to shifts in life history strategy.

Numbers of 15-d-old nestlings were affected by seasonal dynamics in a location-dependent manner. As the breeding season advanced, brood sizes declined in Bermuda but not in Ohio. This result could suggest that the quality of the match between the available resources and the needs of breeding bluebirds is more temporally variable in Bermuda. Abiotic (e.g., weather) and biotic (e.g., insect abundance) factors, either individually or in an interconnected manner, can trigger a mismatch (Blomberg et al. 2012; Burger et al. 2012). Environments likely differ in their proneness to temporal fluctuations in resources: for example, Bermuda, while relatively stable in terms of climate, may turn inhospitable more rapidly than Ohio because of the island's absence of sources of freshwater other than rain. Furthermore, island and continental bluebirds may differ in their ability to cope with resource fluctuations (Crowell and Rothstein 1981). Since feeding rates were unrelated to the location of the population or to the progression of the breeding season, the decline in brood size in Bermuda was apparently not simply a manifestation of reduced parental effort. In theory, intensified parental effort in Bermuda could have offset the decline, but our feeding rate data offer little evidence for such a strategy.

It is worth noting that during the breeding season, days are slightly longer in Ohio than in Bermuda (on the summer solstice, 14.9 h in Ohio, 14.1 h in Bermuda). Thus, on a 24-h basis, parents in Ohio may feed up to 6% more, given a diurnally constant feeding rate that is the same in both populations. This difference could be one mechanism behind the population-specific rates of nestling development (which differed by ~5%). Other variables related to food delivery (e.g., item quality, quantity per visit, etc.) may also underlie population differences and are worth exploring (Burger et al. 2012). Quantifying incubation effort and duration, as well as fledgling success (rather than day 15 as proxies) and survival, will be useful for fleshing out the life history strategies in these populations, and these measures will add valuable context to any island-continent differences among hatchlings. Assaying territoriality (Duckworth 2006) and neophobia (Lendvai et al. 2011) of parent birds will also shed light on their valuation of their current brood.

Conclusions and Conservation Implications

In relation to the population's vulnerable status, Bermuda's Department of Conservation Services identified a need for "in depth [studies] into the ecology and behavior of Bermuda's bluebirds" (Bermuda Biodiversity Project 2007). Our study of-

fers a broad examination of morphological, physiological, and behavioral parameters, which is unmatched among studies comparing island and continental populations and completely lacking for island eastern bluebirds. We clearly demonstrated that bluebirds in Bermuda differed phenotypically from bluebirds in Ohio. Only in some cases did the direction of these differences match our predictions, most notably with the slower rates of development in island nestlings.

There are several key conclusions for biologists interested in applying physiological measures to conservation-related questions and for managers focused on conserving small island populations, including the bluebirds of Bermuda. We identified no clear mismatches in terms of life history and physiology, since, for example, slower development was complemented by more robust immune function, but ecological mismatches may still lead to fewer fledglings in Bermuda. More studies are needed to conclusively identify changes in life history and any related effects and to more thoroughly understand the seasonal patterns in fledgling numbers in Bermuda. Efforts aimed at conserving the bluebirds of Bermuda should focus on the intraseasonal patterns in nestling mortality and, more generally, the survival probabilities of different age classes. Furthermore, conservation planners in Bermuda should consider the consequences of (1) introduced mammalian and avian predators and competitors and their removal and (2) anthropogenic changes to populations of insect prey and possible remediation (e.g., by altering insecticide application regimens). These factors may not only affect survival and mortality rates but also shape associated physiological and life history parameters. Ultimately, our study highlights the value of considering the match between an organism, its environment, and its evolutionary history on a population-specific scale. Without this context, identifying detrimental trends is a more challenging proposition.

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Literature Cited

Adler G.H. and R. Levins. 1994. The island syndrome in rodent populations. Q Rev Biol 69:473–490.

Alcaide M., J.A. Lemus, G. Blanco, J.L. Tella, D. Serrano, J.J. Negro, A. Rodríguez, and M. García-Montijano. 2010. MHC diversity and differential exposure to pathogens in kestrels (Aves: Falconidae). Mol Ecol 19:691–705.

Avery J.D., D.M. Fonseca, P. Campagne, and J.L. Lockwood. 2013. Cryptic introductions and the interpretation of island biodiversity. Mol Ecol 22:2313–2324.

Bates D., M. Maechler, and B. Bolker. 2012. lme4: linear mixed-effects models using S4 classes. http://CRAN.R-project.org/package=lme4.

Bermuda Biodiversity Project. 2007. Species profile for the eastern bluebird. Department of Conservation Services, Ministry of Public Works, Government of Bermuda. http://www.conservation.bm/publications/bermuda-biodiversity-project-species-profiles/bluebird.pdf.

Berzins L.L., H.G. Gilchrist, K.D. Matson, and G. Burness. 2011. Sex-specific effects of increased incubation demand on innate immunity in black guillemots. Physiol Biochem Zool 84:222–229.

Blomberg E.J., J.S. Sedinger, M.T. Atamian, and D.V. Nonne. 2012. Characteristics of climate and landscape disturbance influence the dynamics of greater sage-grouse populations. Ecosphere 3:art55, http://dx.doi.org/10.1890/ES11-00304.1.

Buehler D.M., B.I. Tieleman, and T. Piersma. 2009. Age and environment affect constitutive immune function in red knots (*Calidris canutus*). J Ornithol 150:815–825.

Burger C., E. Belskii, T. Eeva, T. Laaksonen, M. Mägi, R. Mänd, A. Qvarnström, et al. 2012. Climate change, breeding date and nestling diet: how temperature differentially affects seasonal changes in pied flycatcher diet depending on habitat variation. J Anim Ecol 81:926–936.

Burnham K.P. and D.R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Springer, New York.

Case T.J. 1978. A general explanation for insular body size trends in terrestrial vertebrates. Ecology 59:1–18.

Cellier-Holzem E., R. Esparza-Salas, S. Garnier, and G. Sorci. 2010. Effect of repeated exposure to *Plasmodium relictum*

(lineage SGS1) on infection dynamics in domestic canaries. Int J Parasitol 40:1447–1453.

Clegg S.M. and P.F. Owens. 2002. The "island rule" in birds: medium body size and its ecological explanation. Proc R Soc B 269:1359–1365.

Coslovsky M. and H. Richner. 2011. Predation risk affects offspring growth via maternal effects. Funct Ecol 25:878–888.

Crowell K.L. 1962. Reduced interspecific competition among the birds of Bermuda. Ecology 43:75–88.

Crowell K.L. and M.R. Crowell. 1976. Bermuda's abundant, beleaguered birds. Nat Hist 85:48–56.

Crowell K.L. and S.I. Rothstein. 1981. Clutch sizes and breeding strategies among Bermudan and North American passerines. Ibis 123:42–50.

Duckworth R.A. 2006. Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. Behav Ecol 17:1011–1019.

Eaton J.W., P. Brandt, J.R. Mahoney, and J.T. Lee Jr. 1982. Haptoglobin: a natural bacteriostat. Science 215:691–693.

Foster J.B. 1964. Evolution of mammals on islands. Nature 202: 234–235.

Gangoso L., J.M. Grande, J.A. Lemus, G. Blanco, J. Grande, and J.A. Donázar. 2009. Susceptibility to infection and immune response in insular and continental populations of Egyptian vulture: implications for conservation. PLoS ONE 4:e6333.

Goren E., W.A. de Jong, P. Doornenbal, N.M. Bolder, R.W.A.W. Mulder, and A. Jansen. 1988. Reduction of salmonella infection of broilers by spray application of intestinal microflora: a longitudinal study. Vet Q 10:249–255.

Gowaty P.A. and J.H. Plissner. 1998. Eastern bluebird (*Sialia sialis*). In A. Poole and F. Gill, eds. The birds of North America, no. 381. Birds of North America, Philadelphia.

Grant P.R. 1965. The adaptive significance of some size trends in island birds. Evolution 19:355–367.

———. 1998. Evolution on islands. Oxford University Press, Oxford.

Grindstaff J.L. 2008. Maternal antibodies reduce costs of an immune response during development. J Exp Biol 211:654–660.

Hegemann A., K.D. Matson, C. Both, and B.I. Tieleman. 2012. Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. Oecologia 170:605–618.

Horrocks N.P.C., A. Hegemann, K.D. Matson, K. Hine, S. Jaquier, M. Shobrak, J.B. Williams, J.M. Tinbergen, and B.I. Tieleman. 2012*a*. Immune indexes of larks from desert and temperate regions show weak associations with life history but stronger links to environmental variation in microbial abundance. Physiol Biochem Zool 85:504–515.

Horrocks N.P.C., K.D. Matson, M. Shobrak, J.M. Tinbergen, and B.I. Tieleman. 2012*b*. Seasonal patterns in immune indices reflect microbial loads on birds but not microbes in the wider environment. Ecosphere 3:art19, http://dx.doi.org/10.1890/ES11-00287.1.

Horrocks N.P.C., K.D. Matson, and B.I. Tieleman. 2011. Pathogen pressure puts immune defense into perspective. Integr Comp Biol 51:563–576.

Kingsford R.T., J.E.M. Watson, C.J. Lundquist, O. Venter, L. Hughes, E.L. Johnston, J. Atherton, et al. 2009. Major conservation policy issues for biodiversity in Oceania. Conserv Biol 23:834–840.

Kohl K. 2012. Diversity and function of the avian gut microbiota. J Comp Physiol B 182:591–602.

Lack D. 1942. Ecological features of the bird faunas of British small islands. J Anim Ecol 11:9–36.

Lee K.A., M. Wikelski, W.D. Robinson, T.R. Robinson, and K.C. Klasing. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. J Animal Ecol 77: 356–363.

Lendvai Á.Z., V. Bókony, and O. Chastel. 2011. Coping with novelty and stress in free-living house sparrows. J Exp Biol 214:821–828.

Lockwood J.L. and M.P. Moulton. 1994. Ecomorphological pattern in Bermuda birds: the influence of competition and implications for nature preserves. Evol Ecol 8:53–60.

Loehle C. and W. Eschenbach. 2012. Historical bird and terrestrial mammal extinction rates and causes. Divers Distrib 18:84–91.

Lomolino M.V. 2005. Body size evolution in insular vertebrates: generality of the island rule. J Biogeogr 32:1683–1699.

Mathys B.A. and J.L. Lockwood. 2009. Rapid evolution of great kiskadees on Bermuda: an assessment of the ability of the island rule to predict the direction of contemporary evolution in exotic vertebrates. J Biogeogr 36:2204–2211.

Matson K.D. 2006. Are there differences in immune function between continental and insular birds? Proc R Soc B 273: 2267–2274.

Matson K.D., N.P.C. Horrocks, B.I. Tieleman, and E. Haase. 2012*a*. Intense flight and endotoxin injection elicit similar effects on leukocyte distributions but dissimilar effects on plasma-based immunological indices in pigeons. J Exp Biol 215:3734–3741.

Matson K.D., N.P.C. Horrocks, M.A. Versteegh, and B.I. Tieleman. 2012*b*. Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response. Comp Biochem Physiol A 162:7–15.

Matson K.D., R.E. Ricklefs, and K.C. Klasing. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Dev Comp Immunol 29:275–286.

Mauck R.A., K.D. Matson, J. Philipsborn, and R.E. Ricklefs. 2005. Increase in the constitutive innate humoral immune system in Leach's storm-petrel (*Oceanodroma leucorhoa*) chicks is negatively correlated with growth rate. Funct Ecol 19:1001–1007.

Mazerolle M. 2012. AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). http://CRAN.R-project.org/package=AICcmodavg.

Meiri S., N. Cooper, and A. Purvis. 2008. The island rule: made to be broken? Proc R Soc B 275:141–148.

Nice M.M. and W.R.P. Bourne. 1958. The bluebird in Bermuda. Ibis 100:127–128.

Palacios M.G., J.E. Cunnick, D.W. Winkler, and C.M. Vleck. 2007. Immunosenescence in some but not all immune components in a free-living vertebrate, the tree swallow. Proc R Soc B 274:951–957.

Paulay G. 1994. Biodiversity on oceanic islands: its origin and extinction. Am Zool 34:134–144.

Peralta-Sánchez J.M., M. Martín-Vivaldi, A.M. Martín-Platero, M. Martínez-Bueno, M. Oñate, M. Ruiz-Rodríguez, and J.J. Soler. 2012. Avian life history traits influence eggshell bacterial loads: a comparative analysis. Ibis 154:725–737.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar, and R Development Core Team. 2012. nlme: linear and nonlinear mixed effects models. http://CRAN.R-project.org/package = nlme.

Pinkowski B.C. 1975. Growth and development of eastern bluebirds. Bird Band 46:273–289.

Quaye I.K. 2008. Haptoglobin, inflammation and disease. Trans R Soc Trop Med Hyg 102:735–742.

Radersma R., J.M. Tinbergen, and J. Komdeur. 2011. Do brood sex ratio, nestling development and sex affect fledging timing and order? an experimental study on great tits. Anim Behav 81:69–75.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

Ricklefs R.E. 1967. A graphical method of fitting equations to growth curves. Ecology 48:978–983.

Ricklefs R.E. and M. Wikelski. 2002. The physiology/life-history nexus. Trends Ecol Evol 17:462–468.

Sauer J.R. and S. Droege. 1990. Recent population trends of the eastern bluebird. Wilson Bull 102:239–252.

Shawkey M.D., K.L. Kosciuch, M. Liu, F.C. Rohwer, E.R. Loos, J.M. Wang, and S.R. Beissinger. 2008. Do birds differentially distribute antimicrobial proteins within clutches of eggs? Behav Ecol 19:920–927.

Spiller D.A., J.B. Losos, and T.W. Schoener. 1998. Impact of a catastrophic hurricane on island populations. Science 281: 695–697.

Suarez-Rubio M. and J.R. Thomlinson. 2009. Landscape and patch-level factors influence bird communities in an urbanized tropical island. Biol Conserv 142:1311–1321.

van de Crommenacker J., N.P.C. Horrocks, M.A. Versteegh, J. Komdeur, B.I. Tieleman, and K.D. Matson. 2010. Effects of immune supplementation and immune challenge on oxidative status and physiology in a model bird: implications for ecologists. J Exp Biol 213:3527–3535.

Versteegh M.A., I. Schwabl, S. Jaquier, and B.I. Tieleman. 2012. Do immunological, endocrine and metabolic traits fall on a single pace-of-life axis? covariation and constraints among physiological systems. J Evol Biol 25:1864–1876.

Warner R.E. 1968. The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70:101–120.

Wikelski M. and S.J. Cooke. 2006. Conservation physiology. Trends Ecol Evol 21:38–46.

Wikelski M., J. Foufopoulos, H. Vargas, and H. Snell. 2004. Galápagos birds and diseases: invasive pathogens as threats for island species. Ecol Soc 9:5.